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(54) Title: METHODS AND COMPOSITIONS FOR OXYGEN TRANSPORT COMPRISING AN OXYGEN CARRIER AND A CRYSTALLOID IN HYPERTONIC SOLUTION

(57) Abstract: The present invention relates to blood product compositions, and more particularly to compositions comprising a modified hemoglobin and a crystalloid in an aqueous diluent. The present invention also relates to such blood product compositions which are formulated as hypertonic solutions to provide for low volume rapid restoration of hemodynamic parameters in hypovolemic states.

METHODS AND COMPOSITIONS FOR OXYGEN TRANSPORT COMPRISING AN OXYGEN CARRIER AND A CRYSTALLOID IN HYPERTONIC SOLUTION

TECHNICAL FIELD

[0001] The present invention relates to hypertonic blood substitutes comprising an oxygen carrier and a crystalloid in an aqueous solution that provide for low volume rapid restoration of hemodynamic parameters in hypovolemic states.

BACKGROUND OF THE INVENTION

The Circulatory System and the Nature of Hemoglobin

[0002] The blood is the means for delivering nutrients to the tissues and removing waste products from the tissues for excretion. The blood is composed of plasma in which red blood cells (RBCs or erythrocytes), white blood cells (WBCs), and platelets are suspended. Red blood cells comprise approximately 99% of the cells in blood, and their principal function is the transport of oxygen to the tissues and the removal of carbon dioxide therefrom.

[0003] The left ventricle of the heart pumps the blood through the arteries and the smaller arterioles of the circulatory system. The blood then enters the capillaries, where the majority of the exchange of nutrients and cellular waste products occurs. (See, *e.g.*, A. C. Guyton, Human Physiology And Mechanisms Of Disease (3rd. ed.; W. B. Saunders Co., Philadelphia, Pa.), pp. 228-229 (1982)). Thereafter, the blood travels through the venules and veins in its return to the right atrium of the heart. Though the blood that returns to the heart is oxygen-poor compared to that which is pumped from the heart, when at rest, the returning blood still contains about 75% of the original oxygen content.

[0004] The reversible oxygenation function (*i.e.*, the delivery of oxygen) of RBCs is carried out by the protein hemoglobin. In mammals, hemoglobin has a molecular weight of approximately 64,000 daltons and is composed of about 6% heme and 94% globin. In its native form, it contains two pairs of subunits (*i.e.*, it is a tetramer), each containing a heme group and a globin polypeptide chain. In aqueous solution, hemoglobin is present in equilibrium between the tetrameric (MW 64,000) and dimeric forms (MW 32,000); outside of the RBC, the dimers are prematurely excreted by the kidney (plasma half-life of

approximately 2-4 hours). Along with hemoglobin, RBCs contain stroma (the RBC membrane), which comprises proteins, cholesterol, and phospholipids.

Exogenous Blood Products

[0005] Due to the demand for blood products in hospitals and other settings, extensive research has been directed at the development of blood substitutes and plasma expanders. A blood substitute is a blood product that is capable of carrying and supplying oxygen to the tissues. Blood substitutes have a number of uses, including replacing blood lost during surgical procedures and following acute hemorrhage, and for resuscitation procedures following traumatic injury. Plasma expanders are blood substitutes that are administered into the vascular system but are typically not capable of carrying oxygen. Plasma expanders can be used, for example, for replacing plasma lost from burns, to treat volume deficiency shock, and to effect hemodilution (*e.g.*, for the maintenance of normovolemia and to lower blood viscosity). Essentially, blood substitutes can be used for these purposes or any purpose in which banked blood is currently administered to patients. (See, *e.g.*, U.S. Pat. Nos. 4,001,401 to Bonson *et al.*, and 4,061,736 to Morris *et al.*)

[0006] The current human blood supply is associated with several limitations that can be alleviated through the use of an exogenous blood substitute. To illustrate, the widespread availability of safe and effective blood substitutes would reduce the need for banked (allogeneic) blood. Moreover, such blood substitutes would allow the immediate infusion of a resuscitation solution following traumatic injury without regard to cross-matching (as is required for blood), thereby saving valuable time in resupplying oxygen to ischemic tissue. Likewise, blood substitutes can be administered to patients prior to surgery, allowing removal of autologous blood from the patients which could be returned later in the procedure, if needed, or after surgery. Thus, the use of exogenous blood products not only protects patients from exposure to non-autologous (allogeneic) blood, it conserves either autologous or allogeneic (banked, crossmatched) blood for its optimal use.

Limitations of Current Blood Substitutes

[0007] Attempts to produce blood substitutes (sometimes referred to as "oxygen-carrying plasma expanders") have thus far produced products with marginal efficacy or whose manufacture is tedious and expensive, or both. Frequently, the cost of manufacturing such

products is so high that it effectively precludes the widespread use of the products, particularly in those markets where the greatest need exists (*e.g.*, emerging third-world economies).

[0008] Blood substitutes can be grouped into the following three categories: i) perfluorocarbon-based emulsions, ii) liposome-encapsulated hemoglobin, and iii) modified cell-free hemoglobin. As discussed below, none has been entirely successful, though products comprising modified cell-free hemoglobin are thought to be the most promising. Perfluorochemical-based compositions dissolve oxygen as opposed to binding it as a chelate. In order to be used in biological systems, the perfluorochemical must be emulsified with a lipid, typically egg-yolk phospholipid. Though the perfluorocarbon emulsions are inexpensive to manufacture, they do not carry sufficient oxygen at clinically tolerated doses to be effective. Conversely, while liposome-encapsulated hemoglobin has been shown to be effective, it is far too costly for widespread use. (See generally, Winslow, Robert M., "Hemoglobin-based Red Cell Substitutes", Johns Hopkins University Press, Baltimore, 1992).

[0009] Most of the blood substitute products in clinical trials today are based on modified hemoglobin. These products, frequently referred to as hemoglobin-based oxygen carriers (HBOCs), generally comprise a homogeneous aqueous solution of a chemically-modified hemoglobin, essentially free from other red cell residue (stroma). Although stroma-free hemoglobin (SFH) from humans is the most common raw material for preparing a HBOC, other sources of hemoglobin have also been used. For example, hemoglobin can be obtained or derived from animal blood (*e.g.*, bovine or porcine hemoglobin) or from bacteria or yeast or transgenic animals molecularly altered to produce a desired hemoglobin product.

[0010] The chemical modification is generally one of intramolecular cross-linking, oligomerization and/or polymer conjugation to modify the hemoglobin such that its persistence in the circulation is prolonged relative to that of unmodified hemoglobin, and its oxygen binding properties are similar to those of blood. Intramolecular cross-linking chemically binds together subunits of the tetrameric hemoglobin unit to prevent the formation of dimers which, as previously indicated, are prematurely excreted. (See, *e.g.*, U.S. Pat. No. 5,296,465 to Rausch *et al.*)

[0011] The high costs of manufacturing HBOC products have greatly limited their commercial viability. In addition, the present inventors have found that known HBOCs

have a tendency to release excessive amounts of oxygen to the tissues at the arteriole walls rather than the capillaries. This can result in insufficient oxygen available for delivery by the HBOC to the tissues surrounding the capillaries. This is despite the fact that the initial loading of the HBOC with oxygen may be relatively high, even higher than that normally achieved with natural red blood cells.

[0012] In addition, most blood substitutes under development are limited to HBOCs in colloid solutions and solutions having relatively low osmolarity. (See, *e.g.*, U.S. Pat. Nos. 5,814, 601 and 5,661,124.) While such mixtures are sufficient for some blood replacement uses, colloid solutions and low osmolarity crystalloid solutions are not optimal for rapid restoration of intravascular hemodynamic parameters in controlled hemorrhage. Low volume hypertonic crystalloid solutions have been demonstrated superior to colloid solutions in the treatment of controlled hemorrhage. (See, *e.g.*, Fluid Resuscitation: State of the Science for Treating Combat Casualties and Civilian Injuries (The National Academy Press), pp. 103-104 (2000)). Colloid solutions have several other disadvantages, including possible anaphylaxis, inhibition of hemostasis, cost, and large volume requirement in treatment of hemorrhage. (See *e.g.*, *Id.* at 60-61). Hypertonic solutions alone, however, have the disadvantage that they lack significant oxygen-carrying capacity, which may result in inadequate tissue oxygenation. Accordingly, the present invention relates to a blood substitute that comprises an oxygen carrier and a crystalloid which is present in the blood substitute in sufficient quantity to make it hypertonic.

SUMMARY OF THE INVENTION

[0013] The present invention relates to a blood substitute composition containing a modified hemoglobin and a crystalloid in an aqueous solution, such that the composition has an osmolarity greater than 800 mOsm/l and a hemoglobin concentration less than 6 g/dl. For some applications, it is advantageous for the osmolarity to be greater than 2000 mOsm/l.

[0014] The crystalloid may be sodium chloride, although any physiologically acceptable crystalloid can be used. The modified hemoglobin may be native hemoglobin or may be produced recombinantly, and is preferably a polyalkylene oxide-hemoglobin conjugate. Other aspects of the present invention are described throughout the specification.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figure 1 depicts the mean arterial blood pressure during a resuscitation study as described in Example 1.

[0016] Figure 2 depicts the return of lactic and levels in the same study.

[0017] Figure 3 depicts the return of base excess levels in the same study.

DESCRIPTION OF THE INVENTION

[0018] The present invention is directed to hypertonic aqueous solutions of an oxygen carrying component and a crystalloid component that are useful as a blood substitute. For certain applications, such as the rapid restoration of hemodynamic parameters following severe blood loss, the compositions overcome the less than optimal oxygen delivery characteristics of previous blood substitutes. They may therefore be a safer and more effective alternative to other types of blood substitutes, particularly in situations where low volume replacement with rapid restoration of hemodynamic parameters is desirable.

Definitions

[0019] To facilitate understanding of the invention set forth in the disclosure that follows, a number of terms are defined below.

[0020] The term "hemoglobin" refers generally to the protein contained within red blood cells that transports oxygen. Each molecule of hemoglobin has 4 subunits, 2 α chains and 2 β chains, which are arranged in a tetrameric structure. Each subunit also contains one heme group, which is the iron-containing center that binds oxygen. Thus, each hemoglobin molecule can bind 4 oxygen molecules.

[0021] The term "modified hemoglobin" includes, but is not limited to, hemoglobin altered by a chemical reaction such as intra- and inter-molecular cross-linking, genetic manipulation, polymerization, and/or conjugation to other chemical groups (*e.g.*, polyalkylene oxides, for example polyethylene glycol, or other adducts such as proteins, peptides, carbohydrates, synthetic polymers and the like). In essence, hemoglobin is "modified" if any of its structural or functional properties have been altered from its native state. As used herein, the term "hemoglobin" by itself refers both to native, unmodified, hemoglobin, as well as modified hemoglobin.

[0022] The term "surface-modified hemoglobin" is used to refer to hemoglobin described above to which chemical groups such as dextran or polyalkylene oxide have been attached, most usually covalently.

[0023] The term "stroma-free hemoglobin" refers to hemoglobin from which all red blood cell membranes have been removed.

[0024] The term "perfluorocarbons" refers to synthetic, inert, molecules that contain fluorine atoms, and that consist entirely of halogen (Br, F, Cl) and carbon atoms. In the form of emulsions, they are under development as blood substances, because they have the ability to dissolve many times more oxygen than equivalent amounts of plasma or water.

[0025] The term "plasma expander" refers to any solution that may be given to a subject to treat blood loss.

[0026] The term "oxygen carrying capacity", or simply "oxygen capacity" refers to the capacity of a blood substitute to carry oxygen, but does not necessarily correlate with the efficiency in which it delivers oxygen. Oxygen carrying capacity is generally calculated from hemoglobin concentration, since it is known that each gram of hemoglobin binds 1.34 ml of oxygen. Thus, the hemoglobin concentration in g/dl multiplied by the factor 1.34 yields the oxygen capacity in ml/dl. Hemoglobin concentration can be measured by any known method, such as by using the B-Hemoglobin Photometer (HemoCue, Inc., Angelholm, Sweden). Similarly, oxygen capacity can be measured by the amount of oxygen released from a sample of hemoglobin or blood by using, for example, a fuel cell instrument (*e.g.*, Lex-O_2-Con; Lexington Instruments).

[0027] The term "oxygen affinity" refers to the avidity with which an oxygen carrier such as hemoglobin binds molecular oxygen. This characteristic is defined by the oxygen equilibrium curve which relates the degree of saturation of hemoglobin molecules with oxygen (Y axis) with the partial pressure of oxygen (X axis). The position of this curve is denoted by the value, P50, the partial pressure of oxygen at which the oxygen carrier is half-saturated with oxygen, and is inversely related to oxygen affinity. Hence the lower the P50, the higher the oxygen affinity. The oxygen affinity of whole blood (and components of whole blood such as red blood cells and hemoglobin) can be measured by a variety of methods known in the art. (See, *e.g.*, Winslow *et al.*, J. Biol. Chem. 252(7):2331-37 (1977)). Oxygen affinity may also be determined using a commercially available HEMOX™ TM Analyzer (TCS Scientific Corporation, New Hope, Pennsylvania). (See,

e.g., Vandegriff and Shrager in Methods in Enzymology (Everse *et al.*, eds.) 232:460 (1994)).

[0028] The terms "hypertonic" and "hyperosmolar" means an osmolarity greater than 800 mOsm/l, which is the average osmolarity of whole blood. The phrase "highly hypertonic" refers to solutions with an osmolarity greater than 2000 mOsm/l. Osmolarity may be measured by any suitable technique, such as in a Wescor instrument (Ontario, Canada).

[0029] The term "oxygen-carrying component" refers broadly to a substance capable of carrying oxygen in the body's circulatory system and delivering at least a portion of that oxygen to the tissues. In preferred embodiments, the oxygen-carrying component is native or modified hemoglobin, and is also referred to herein as a "hemoglobin based oxygen carrier", or "HBOC".

[0030] The term "hemodynamic parameters" refers broadly to measurements indicative of blood pressure, flow and volume status, including measurements such as blood pressure, cardiac output, right atrial pressure, and left ventricular end diastolic pressure.

[0031] The term "crystalloid" refers to small molecules (usually less than 10 Å) such as salts, sugars, and buffers. Unlike colloids, crystalloids do not contain any oncotically active components and therefore leave the circulation very quickly.

[0032] The term "colloid", in contrast to "crystalloid" refers to larger molecules (usually greater than 10 Å) that do not freely pass through biological membranes and includes proteins such as albumin and gelatin, as well as starches such as pentastarch and hetastarch.

[0033] The term "colloid oncotic pressure" or "colloid osmotic pressure" refers to the propensity of colloids to remain in the interavascular space for prolonged periods of time drawing water from the interstitial and intracellular spaces into the intravascular space.

[0034] Finally, the term "mixture" refers to a mingling together of two or more substances without the occurrence of a reaction by which they would lose their individual properties; the term "solution" refers to a liquid mixture; the term "aqueous solution" refers to a solution that contains some water and may also contain one or more other liquid substances with water to form a multi-component solution; the term "approximately" refers to the actual value being within a range, *e.g.* 10%, of the indicated value. The meaning of other terminology used herein should be easily understood by someone of reasonable skill in the art.

The Nature of Oxygen Delivery and Consumption

[0035] Although the successful use of the compositions and methods of the present invention do not require comprehension of the underlying mechanisms of oxygen delivery and consumption, basic knowledge regarding some of these putative mechanisms may assist in understanding the discussion that follows. It has generally been assumed that the capillaries are the primary conveyors of oxygen to the tissue. However, regarding tissue at rest, current findings indicate that there is approximately an equipartition between arteriolar and capillary oxygen release. That is, hemoglobin in the arterial system is believed to deliver approximately one third of its oxygen content in the arteriolar network and one-third in the capillaries, while the remainder exits the microcirculation via the venous system.

[0036] The arteries themselves are sites of oxygen utilization. For example, the artery wall requires energy to effect regulation of blood flow through contraction against vascular resistance. Thus, the arterial wall is normally a significant site for the diffusion of oxygen out of the blood. However, current oxygen-delivering compositions (*e.g.*, HBOCs) may release too much of their oxygen content in the arterial system, and thereby induce an autoregulatory reduction in capillary perfusion. Accordingly, the efficiency of oxygen delivery of a blood substitute may actually be hampered by having too much oxygen or too low an oxygen affinity.

[0037] The rate of oxygen consumption by the vascular wall, *i.e.*, the combination of oxygen required for mechanical work and oxygen required for biochemical synthesis, can be determined by measuring the gradient at the vessel wall. See, *e.g.*, Winslow, *et al.*, in "Advances in Blood Substitutes" (1997), Birkhauser, ed., Boston, MA, pages 167-188. Present technology allows accurate oxygen partial pressure measurements in a variety of vessels. The measured gradient is directly proportional to the rate of oxygen utilization by the tissue in the region of the measurement. Such measurements show that the vessel wall has a baseline oxygen utilization which increases with increases in inflammation and constriction, and is lowered by relaxation.

[0038] The vessel wall gradient is inversely proportional to tissue oxygenation. Vasoconstriction increases the oxygen gradient (tissue metabolism), while vasodilation lowers the gradient. Higher gradients are indicative of the fact that more oxygen is used by

the vessel wall, while less oxygen is available for the tissue. The same phenomenon is believed to be present throughout the microcirculation.

Oxygen-Carrying Component

[0039] In preferred embodiments, the oxygen carrier (*i.e.*, the oxygen-carrying component) is a hemoglobin-based oxygen carrier, or HBOC. The hemoglobin may be either native (unmodified); subsequently modified by a chemical reaction such as intra- or inter-molecular cross-linking, polymerization, or the addition of chemical groups (*e.g.*, polyalkylene oxides, or other adducts); or it may be recombinantly engineered. Human alpha- and beta-globin genes have both been cloned and sequenced. Liebhaber, *et al.*, P.N.A.S. 77: 7054-7058 (1980); Marotta, *et al.*, J. Biol. Chem. 353: 5040-5053 (1977) (beta-globin cDNA). In addition, many recombinantly produced modified hemoglobins have now been produced using site-directed mutagenesis, although these "mutant" hemoglobin varieties were reported to have undesirably high oxygen affinities. See, *e.g.*, Nagai, *et al.*, P.N.A.S. 82: 7252-7255 (1985).

[0040] The present invention is not limited by the source of the hemoglobin. For example, the hemoglobin may be derived from animals and humans. Preferred sources of hemoglobin are humans, cows and pigs. In addition, hemoglobin may be produced by other methods, including chemical synthesis and recombinant techniques. The hemoglobin can be added to the blood product composition in free form, or it may be encapsulated in a vessicle, such as a synthetic particle, microballoon or liposome. The present invention also contemplates the use of other means for oxygen delivery that do not entail hemoglobin or modified hemoglobin, such as the fluorocarbon emulsions.

[0041] The preferred oxygen-carrying components of the present invention should be stroma free and endotoxin free. Representative examples of oxygen-carrying components are disclosed in a number of issued United States Patents, including U.S. Pat. No. 4,857,636 to Hsia; U.S. Pat. No. 4,600,531 to Walder, U.S. Pat. No. 4,061,736 to Morris *et al.*; U.S. Pat. No. 3,925,344 to Mazur; U.S. Pat. No. 4,529,719 to Tye; U.S. Pat. No. 4,473,496 to Scannon; 4,584,130 to Bocci *et al.*; U.S. Pat. No. 5,250,665 to Kluger *et al.*; U.S. Pat. No. 5,028,588 to Hoffman *et al.*; and U.S. Pat. No. 4,826,811 and U.S. Pat. No. 5,194,590 to Sehgal *et al.*

[0042] However, as discussed above, the present inventors theorize that blood substitutes with lower oxygen affinities may trigger autoregulatory events that prevent oxygen

delivery to the tissues via microcapillary circulation. Accordingly, using the experimental models described in Winslow, supra, it has been determined that, for some applications an HBOC with an oxygen affinity less than that of SFH is desired. This finding is contrary to conventional teachings in the field.

[0043] There are many different scientific approaches to manufacturing HBOCs with high oxygen affinity (*i.e.* those with P50s less than SFH). For example, studies have identified the amino acid residues that play a role in oxygen affinity, and thus site-directed mutagenesis can now be easily carried out to manipulate oxygen affinity to a desired level. See, *e.g.*, U.S. Patent No. 5,661,124. Many other approaches are discussed in U.S. Patent No. 6,054,427.

Modifications of the Oxygen-Carrying Component

[0044] In a preferred embodiment, the oxygen-carrying component is modified hemoglobin. A preferred modification to hemoglobin is "surface-modification", *i.e.* covalent attachment of chemical groups to the exposed amino acid side chains on the hemoglobin molecule. Most commonly, the chemical group attached to the hemoglobin is polyethylene glycol (PEG), because of its pharmaceutical acceptability and commercial availability. PEGs are polymers of the general chemical formula $H(OCH_2CH_2)_nOH$, where n is greater than or equal to 4. PEG formulations are usually followed by a number that corresponds to their average molecular weight. For example, PEG-200 has an average molecular weight of 200 and may have a molecular weight range of 190-210. PEGs are commercially available in a number of different forms, and in many instances come preactivated and ready to conjugate to proteins.

[0045] The number of PEGs to be added to the hemoglobin molecule may vary, depending on the size of the PEG. However, the molecular size of the resultant modified hemoglobin should be sufficiently large to avoid being cleared by the kidneys to achieve the desired half-life. Blumenstein, *et al.*, determined that this size is achieved above 84,000 molecular weight. (Blumenstein, *et al.*, in "Blood Substitutes and Plasma Expanders", Alan R. Liss, editors, New York, New York, pages 205-212 (1978).) Therein, the authors conjugated hemoglobin to dextran of varying molecular weight. They reported that a conjugate of hemoglobin (with a molecular weight of 64,000) and dextran (having a molecular weight of 20,000) "was cleared slowly from the circulation and negligibly through the kidneys", but increasing the molecular weight above 84,000 did not alter the clearance curves.

Accordingly, as determined by Blumenstein, *et al.*, it is preferable that the HBOC have a molecular weight of at least 84,000.

Crystalloid component

[0046] In the present invention, the blood substitute comprises a crystalloid. The crystalloid component can be any crystalloid which, in the form of the blood substitute composition, is preferably capable of achieving an osmolarity greater than 800 mOsm/l, *i.e.* it makes the blood substitute "hypertonic". Examples of suitable crystalloids and their concentrations in the blood substitute include, *e.g.*, 3% NaCl, 7% NaCl, 7.5% NaCl, and 7.5% NaCl in 6% dextran. More preferably, the blood substitute has an osmolarity of between 800 and 2400 mOsm/l. The use of recombinantly produced hemoglobins in solutions with an osmolality between 300 - 800 mOsm/l that further comprise a colloid (*i.e.* a molecule less diffusible than dextrose) have been previously reported. See, *e.g.*, U.S. Patent No. 5,661,124. However, this patent teaches away from producing blood substitutes with osmolalities above 800, and suggests that the hemoglobin concentration should be between 6-12 g/dl. In contrast, the oxygen carrying efficiency of compositions of the present invention permit lower concentrations of hemoglobin to be used, such as less than 6 g/dl or even less than 4 g/dl.

[0047] When the blood substitute further comprises a crystalloid and is hypertonic, the compositions of present invention may provide improved functionality for rapid recovery of hemodynamic parameters over other blood substitute compositions, which include a colloid component. Small volume highly hypertonic crystalloid infusion (*e.g.*, 1-10 ml/kg) provides significant benefits in the rapid and sustained recovery of acceptable hemodynamic parameters in controlled hemorrhage. (See, *e.g.*, Przybelski, R. J., E. K. Daily, and M. L. Birnbaum, "The pressor effect of hemoglobin -- good or bad?" In Winslow, R. M., K. D. Vandegriff, and M. Intaglietta, eds. Advances in Blood Substitutes. Industrial Opportunities and Medical Challenges. Boston, Birkhäuser (1997), 71-85). Hypertonic crystalloid solutions alone, however, do not adequately restore cerebral oxygen transport. See D. Prough, et al., Effects of hypertonic saline versus Ringer's solution on cerebral oxygen transport during resuscitation from hemorrhagic shock J. Neurosurg. 64:627-32 (1986).

Formulation

[0048] The blood substitutes of the present invention are formulated by mixing the oxygen carrier and other optional excipients with a suitable diluent. Although the concentration of the oxygen carrier in the diluent may vary according to the application, and in particular based on the expected post-administration dilution, in preferred embodiments, because of the other features of the compositions of the present invention that provide for enhanced oxygen delivery and therapeutic effects, it is usually unnecessary for the concentration to be above 6 g/dl, and is more preferably between 0.1 to 4 g/dl.

Clinical Applications

[0049] The methods and compositions of the present invention are useful in a variety of different applications, such as hemodilution, trauma, septic shock, ischemia, cancer, anemia, cardioplegia, hypoxia and organ perfusion. These and other applications are discussed extensively in U.S. Patent No. 6,054,427.

[0050] All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in hematology, surgical science, transfusion medicine, transplantation, or any related fields are intended to be within the scope of the following claims.

Example 1

Resuscitation Experiment With Hypertonic PEG-Hb

[0051] PEG modified hemoglobin was prepared from human hemoglobin isolated from outdated blood. The hemoglobin was first thiolated and then reacted with maleimide-activated PEG, 5,000 Daltons. Sprague-Dawley rats (n=2) were shock-induced for thirty minutes essentially as described in U.S. Patent No. 6,054,427. Essentially, maintenance of the blood pressure at 40 mm/Hg for 30 minutes results in severe shock as indicated by an

increase in lactic acid levels and excess of base. Thereafter, PEG modified hemoglobin in a hypertonic saline solution (7.5% sodium chloride) was infused to a level of 20% of the original blood volume. The results are shown in Figures 1, 2 and 3. Figure 1 shows the return of a normal level of mean arterial blood pressure following administration of the blood substitute. Figure 2 shows the return of lactic acid levels post-administration, and Figure 3 shows the return of base excess levels.

Claims

We claim:

1. A blood substitute composition comprising a modified hemoglobin, a crystalloid and an aqueous diluent, wherein said composition has an osmolarity greater than 800 mOsm/l and a hemoglobin concentration less than 6 g/dl.

2. The blood substitute composition of claim 1, wherein said composition has an osmolarity greater than 2000 mOsm/l.

3. The blood substitute composition of claim 1, wherein said crystalloid comprises sodium chloride.

4. The blood substitute composition of claim 1, wherein said modified hemoglobin comprises a polyalkylene oxide-hemoglobin conjugate.

5. The blood substitute composition of claim 1, wherein said hemoglobin is selected from the group consisting of native hemoglobin and recombinant hemoglobin.

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(54) Title: METHODS AND COMPOSITIONS FOR OXYGEN TRANSPORT COMPRISING AN OXYGEN CARRIER AND A CRYSTALLOID IN HYPERTONIC SOLUTION

(57) Abstract: The present invention relates to blood product compositions, and more particularly to compositions comprising a modified hemoglobin and a crystalloid in an aqueous diluent. The present invention also relates to such blood product compositions which are formulated as hypertonic solutions to provide for low volume rapid restoration of hemodynamic parameters in hypovolemic states.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,661,124 A (HOFFMAN et al.) 26 August 1997.	1-5

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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